A Modified Procedure for the Microdetermination of Citric Acid

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During the course of an investigation of the citric acid content of bones and teeth it became necessary to determine amounts of the acid varying from 20 to 100 μg. The method of Weil-Malherbe & Bone (1949) was employed and found to be very reliable for 0·2–1·0 mg. citric acid, but with smaller amounts the results were very erratic and recoveries were low and variable. A detailed study of the method applied to the estimation of quantities up to 100 μg. was undertaken as a result of which certain modifications are suggested to increase the sensitivity and precision of the method, while at the same time giving increased speed and simplicity of working. The modified technique applies equally well to the determination of larger quantities of citric acid, up to 1 mg.

The method of Weil-Malherbe & Bone is based on the oxidation of citric acid to acetonedicarboxylic acid in the presence of sulphuric acid and the conversion of this compound to pentabromoacetone. The most important respect in which it differs from earlier methods is in the use of vanadic acid as oxidizing agent instead of permanganate. Bromination of the acetonedicarboxylic acid is effected by bromine water, excess of which is removed with sodium thiosulphate. The pentabromoacetone is then extracted with light petroleum, a portion of which is shaken with sodium sulphide solution. The intensity of the yellow colour produced in the aqueous phase is measured with a suitable photoelectric instrument.

The source of error was eventually traced to an excess of thiosulphate, and, in view of the impossibility of avoiding a local excess, it was decided to use another reducing agent. Ferrous sulphate, recommended by Pucher, Sherman & Vickery (1936) and by Taussky (1949) was finally chosen because a slight excess of this reagent does not interfere. A bromide-bromate mixture was substituted for saturated bromine water and the ammonium vanadate incorporated in this solution.

In the original method the tubes containing the citric acid solution are cooled to room temperature after adding 27 N-sulphuric acid, the bromine water and vanadate are then added separately and the tubes heated in a water bath at 50° for 20 min. In the modification suggested here the bromide-bromate-vanadate mixture is added immediately after the sulphuric acid, when the temperature reaches a maximum of approx. 50°. A slightly higher yield of pentabromoacetone is obtained if the reaction is allowed to take place at room temperature, as Goldberg & Bernheim (1944) have shown, but this advantage is outweighed by the delay involved in cooling the tubes after the addition of the sulphuric acid.

METHOD

Reagents

Sulphuric acid. 27 N-H_2SO_4 was prepared by diluting 750 ml. of the pure concentrated acid to 1 l.

Bromide-bromate-vanadate solution. This contained 19·836 g. KBr, 5·440 g. $KBrO_3$ and 12·000 g. NH_4VO_3 in 1 l. This solution is stable for at least 3 months.

Ferrous sulphate solution. 22·0% (w/v) $FeSO_4.7H_2O$ (A.R.) in N-H_2SO_4. This solution is stored under hydrogen.

Sodium sulphide (A.R.). 2% solution freshly prepared.

Sodium sulphate. Anhydrous.

Light petroleum, b.p. 80–100°. A.R. aromatic-free grade was found satisfactory.

Citric acid stock solution. 0·2187 g. of the crystalline acid ($C_6H_8O_7.H_2O$)/l. in N-H_2SO_4. 25 ml. of this solution diluted to 250 ml. with N-H_2SO_4 contain 100 μg. anhydrous citric acid/5 ml.

Procedure

5 ml. 27 N-H_2SO_4 are added to 5 ml. of the citric acid solution contained in a test tube (150 × 24 mm.), fitted with a ground-glass stopper and, after mixing, 5 ml. of the bromide-bromate-vanadate reagent are run in from a pipette. The tube is stoppered, the contents mixed and allowed to stand for 20–30 min. Ferrous sulphate solution (2 ml.) is then added from a burette connected to a storage bottle and, after mixing, the tube is allowed to stand for 10 min. with occasional shaking to facilitate the removal of Br_2 from the air space. When all the Br_2 has been destroyed the solution is emerald green in colour, probably due to the formation of the hypovanadate ion, $V_2O_6^{4-}$. Light petroleum (6 ml.) is added and the tube is shaken for 1 min. either mechanically or by hand. The lower aqueous layer is then sucked off as completely as possible by means of a 20 or 25 ml. fine-tipped pipette fitted with a rubber extension tube for convenient suction. Sufficient anhydrous Na_2SO_4 is added to absorb the remaining drops of acid and prevent them from running out, and the petroleum is poured into a dry test tube. A 5 ml. portion is pipetted into a dry stoppered tube (125 × 18 mm.) and shaken by hand for 1 min. with Na_2S solution; the volume of this solution used was usually 3 ml. for a Spekker absorptiometer, and 4 ml. for a Unicam spectrophotometer. The yellow aqueous layer is sucked off with a dry pipette and filtered on a dry paper (Whatman no. 1, 4·25 cm.) into a test tube or colorimeter tube.

The absorption of the solution is measured with a Spekker absorptiometer using a 2 cm. microcell and Ilford no. 601 (spectrum violet) filters, or with a Unicam spectrophotometer set at 450 mμ., using an 0·5 in. tube. If the latter is used the colours are developed three or four at a time and the colour intensification of each solution is followed by taking readings of each in turn until steady maxima are obtained. With a Spekker it is more convenient to measure the light absorption of a batch of eight to ten solutions from 15 to 30 min. after colour development. Colour fading is not serious over this period in subdued light.

If the original solutions contain very small amounts of citric acid 10 ml. may be taken, and double quantities of H_2SO_4, bromide reagent and $FeSO_4$ added; 6 ml. of petroleum are still used for extraction. Tubes 150 × 28 mm. are a suitable size.

With these modifications it is possible to complete up to thirty determinations (including standards) in a normal working day.

Precautions. Care must be taken to avoid the two main sources of error stressed by Weil-Malherbe & Bone (1949), namely the incomplete reduction of free Br_2 and the contamination of the petroleum extract with acid. Incomplete removal of Br_2 may be caused either by deterioration of the $FeSO_4$ during storage or by inadequate shaking, and is easily detected by smelling before the addition of the petroleum. Acid contamination should not occur if sufficient Na_2SO_4 is added and if all glassware with which the Na_2S comes in contact is scrupulously clean.

Colour stability. The yellow colour is somewhat unstable. Initially its intensity increases gradually, reaching a maximum 12–15 min. after the end of the shaking period. Thereafter it slowly fades, particularly if exposed to strong light. In their discussion of colour stability Weil-Malherbe & Bone emphasize the importance of temperature on the intensity of the yellow colour and this has been confirmed. Hence a calibration curve should be prepared with each batch of determinations.

Accuracy. The calibration curve with 20–200 μg. citric acid is a straight line, but it seldom runs exactly through the origin. Data showing the degree of accuracy of the method compared with the results reported by Weil-Malherbe & Bone are given in Table 1. Recoveries of known quantities of citric acid added to different amounts of a dilute HCl extract of bone, the citric acid content of which was simultaneously estimated, were studied. The results of a particular experiment are given in Table 2.

Table 1. *Analysis of pure citric acid solutions*

(Using a Unicam spectrophotometer set at 450 mμ. with 0·5 in. tubes and 4 ml. Na_2S solution for colour development.)

Anhydrous citric acid (μg.)	No. of determinations	Coefficient of variation
20	15	3·51
50	15	1·64
100	12	1·87 (4·14)*
200	14	1·28 (1·57)*

* Corresponding figures calculated from the data presented by Weil-Malherbe & Bone (1949).

Table 2. *Recoveries of pure citric acid added to dilute hydrochloric acid extracts of bone*

	Citric acid (μg.)			
Bone extract	Added	Total found	Recovery	Recovery (%)
58·3	12·0	70·6	12·3	102·5
58·3	24·0	82·0	23·7	98·8
58·3	48·0	106·4	48·1	100·2
87·5	16·0	103·0	15·5	96·9

SUMMARY

A modified procedure for the estimation of 20–200 μg. of citric acid is described which is well adapted to the routine examination of large numbers of samples.

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